Downregulation of Pgc-1α expression by tea leaves and their by-products.

AUTHOR(S):

Shibuya, Erika; Murakami, Masaru; Kondo, Makoto; Kamei, Yasutomi; Tomonaga, Shozo; Matsui, Tohru; Funaba, Masayuki

CITATION:

Shibuya, Erika ...[et al]. Downregulation of Pgc-1α expression by tea leaves and their by-products.. Cell biochemistry and function 2013, 32(3): 236-240

ISSUE DATE:

2013-09-20

URL:

http://hdl.handle.net/2433/198734

Down-regulation of Pgc-1α expression by tea leaves and their byproducts

Erika Shibuya[1], Masaru Murakami[2], Makoto Kondo[3], Yasutomi Kamei[4], Shozo Tomonaga[1], Tohru Matsui[1] and Masayuki Funaba[1]*

[1]Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

[2]Laboratory of Molecular Biology, Azabu University School of Veterinary Medicine, Sagamihara 252-5201, Japan

[3]Graduate School of Bioresources, Mie University, Tsu 514-8507, Japan

[4]Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto 606-8522

Running title: Regulatory expression of Pgc-1α by tea leaf

*Corresponding author: Masayuki Funaba, Ph.D.
Division of Applied Biosciences
Graduate School of Agriculture
Kyoto University
Kitashirakawa Oiwakecho, Kyoto 606-8502, Japan
Tel.: +81-75-753-6055
Fax: +81-75-753-6344
E-mail: mfunaba@kais.kyoto-u.ac.jp

Abstract

Previous studies indicate that muscle Pgc-1α expression governs the proportion of muscle-fiber types. As a first step in using diet to manipulate the proportion of muscle-fiber types using Pgc-1α expression, the present study investigates the modulation of Pgc-1α expression by feedstuffs. A luciferase-based Pgc-1α reporter construct (Pgc-1α(-2553)-luc) that contains the mouse Pgc-1α promoter (-2553 to +78 bp) was prepared. A screen of ethanol extracts from 33 feedstuffs indicated that oolong tea and roasted green tea extracts decreased Pgc-1α(-2553)-luc expression in C2C12 myoblasts. The transcriptional repression of Pgc-1α by tea leaf extracts was reproduced in hepatic HepG2 cells. We further examined the effects of the alcohol extracts of tea waste and its silage on Pgc-1α transcription; the tea waste silage extract inhibited Pgc-1α transcription. Treatment with the extracts of raw tea leaves, tea waste and tea waste silage effectively decreased Pgc-1α mRNA levels during myogenesis of myosatellite cells. The present results suggest that tea leaves and their byproducts could be used to modulate proportions of muscle-fiber types.

KEY WORDS: Pgc-1α; skeletal muscle; tea leaf; muscle fiber type; feedstuffs

INTRODUCTION

Pparγ co-activator-1α (Pgc-1α) was originally identified as a co-activator of Pparγ; Pgc-1α enhances the expression of the Ucp1 gene, which encodes an uncoupling protein found in the mitochondria of brown fat depots and is essential to generating heat by non-shivering thermogenesis.[1] Subsequent analyses in cultured cells and transgenic mice have established that the biological function of Pgc-1α is to regulate mitochondrial oxidative metabolism as well as mitochondrial biogenesis in diverse cell types.[2-4] In skeletal muscle, Pgc-1α expression is enhanced by exercise in humans and rodents[5-7] and is involved in the maintenance of muscle function; transgenic mice with enhanced Pgc-1α expression in skeletal muscles preserve muscle integrity and function during aging. This phenotype is related to the maintenance of the activity of enzymes involved in oxidative phosphorylation, the reduction of apoptosis, autophagy and proteasomal degradation.[8] In addition, the transgenic mice exhibited resistance to age-related obesity and increased insulin sensitivity.[8] In contrast, reduced muscle function and exercise capacity were detected in skeletal muscle-specific Pgc-1α knock-out mice.[9]

Metabolic changes related to muscle Pgc-1α expression can be partly explained by the modulation of muscle-fiber types. Muscle fibers are divided into two types, slow-twitch and fast-twitch. Reddish slow-twitch myofibers contain a high number of mitochondria and use oxidative metabolism as their primary energy source, whereas whitish fast-twitch myofibers contain more glycogens and predominantly use glycolytic metabolism.[10] Mouse muscles forced to express Pgc-1α state contained more slow-twitch muscle fibers,[11] whereas gene targeting of skeletal muscle Pgc-1α increased expression of fast-twitch muscle fiber-specific myosin heavy chain (Myhc) 2b and decreased the expression of Myhc 1, which is slow-twitch muscle fiber-specific.[9]

Pgc-1α activity is closely linked to its expression level because it is primarily regulated

at the transcriptional level. MEF2 activates Pgc-1α gene transcription, and MEF2 repression decreases Pgc-1α mRNA levels, leading to the down-regulation of genes involved in fatty acid oxidation in the heart.[12] In addition, selective expression of constitutively active CAMK IV in skeletal muscle increases Pgc-1α transcription and mRNA levels and mitochondrial DNA copy number.[13] Therefore, Pgc-1α activity can be potentially monitored by evaluation of its mRNA levels. The objective of this study is to identify feedstuffs that affect Pgc-1α expression. We screened for Pgc-1α expression using luciferase under the control of the Pgc-1α promoter as a reporter. Here, we show that tea leaves and their byproducts contain factors that down-regulate Pgc-1α expression through transcriptional inhibition.

MATERIALS AND METHODS

Cell culture

Animal experiments were approved by the Kyoto University Animal Experiment Committee. Myosatellite cells were isolated from rat plantaris muscles; male Wistar rats weighing 200 to 300 g were sacrificed by exsanguination under isoflurane anesthesia, and the plantaris muscle was collected. Muscles were washed with phosphate-buffered saline and minced with scissors in digestion buffer containing 0.1% (w/v) collagenase I (Wako, Osaka, Japan) and 1,000 U/ml dispase (Invitrogen, Grand Island, NY, USA) in 140 mM NaCl, 5.4 mM KCl, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 0.81 mM $MgSO_4 \cdot 7H_2O$, 1.3 mM $CaCl_2$, 4.2 mM $NaHCO_3$, and 5.6 mM glucose supplemented with 10 U/mL penicillin, 100 μg/mL streptomycin and 2.5 μg/mL amphotericin B, followed by enzyme digestion shaking at 100 rpm for 60 min at 37°C. After filtering through 50 μm nylon mesh, cells were washed with Dulbecco's modified Eagle's medium (DMEM) with 10% FBS and antibiotics twice and seeded on cell culture dishes. On day one post confluence, cells were differentiated into myotubes using

differentiation medium, i.e., DMEM with 2% horse serum and antibiotics. Ethanol extracts of feedstuffs (20 μg/mL) or forskolin (10 μM) were simultaneously added to the differentiation medium. Cells were harvested 12 days post-differentiation to analyze Pgc-1α expression.

C2C12 cells, COS7 cells and HepG2 cells were cultured in DMEM with 10% FBS and antibiotics. The DMEM used for C2C12 cells contained 1 g/L of glucose, whereas the medium used for COS7 and HepG2 cells contained 4.5 g/L of glucose. For luciferase-based reporter assays, plasmid vectors were transiently transfected using Lipofectamine LTX reagent (Invitrogen) for C2C12 cells or PolyFect transfection reagent (Qiagen, Valencia, CA, USA) for COS7 and HepG2 cells according to the manufacturers' protocol. At 24 h post transfection, cells were treated with or without the extracts from feedstuffs (20 μg/mL) or forskolin (10 μM) for 24 h.

Preparation of ethanol extract

A total of 41 feedstuffs were used. Wet tea wastes were obtained from a local beverage company that produces canned and bottled tea drinks. Tea waste was ensiled in laboratory silos using the method described by Nishino et al.[14] Two grams of food was mixed with 40 mL of ethanol and vigorously shaken for 60 min at room temperature followed by filtration through filter paper. For tea waste and its silage, the ethanol extraction was performed after freeze drying the samples. The ethanol extracts were concentrated to ~20 mg ethanol extract / mL using a centrifugal evaporator (RD-400, Yamato Scientific, Tokyo, Japan). The samples were stored under N_2 gas at -20°C until analysis.

RNA isolation and RT-quantitative PCR

Total RNA isolation from myosatellite cells and cDNA synthesis were performed using

TRIZOL (Invitrogen) and the ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan) respectively according to the manufacturers' protocols. The cDNA was reverse-transcribed from 5 ng of total RNA and was used as a template for RT-quantitative PCR (RT-qPCR) as described previously.[15] The oligonucleotide primers were 5'-TGTGGAACTCTCTGGAACTGC-3' and 5'-GCCTTGAAAGGGTTATCTTGG-3' for Pgc-1α and 5'-CTAAGGCCAACCGTGAAAAG-3' and 5'-ACCAGAGGCATACAGGGACA-3' for β-actin. The Ct values were determined, and the abundance of gene transcripts was analyzed using the ΔΔCt method using β-actin as the normalization gene.

Plasmids and reporter assays

The DNA fragment encompassing -2554 bp and +79 bp of the mouse Pgc-1α promoter was amplified and cloned into the pGL4 basic vector containing the firefly luciferase reporter gene (Pgc-1α(-2553)-luc); nt +1 is the transcription initiation site. The reporter construct contains the promoter region used in study by Amat et al.,[16] who showed transcriptional regulation of Pgc-1α by MyoD. The coding region of mouse MyoD was cloned into pcDNA3. The product was verified by nucleotide sequencing. Cells were transiently transfected with the indicated expression vectors: Pgc-1α(-2553)-luc and a plasmid expressing Renilla luciferase under the control of thymidine kinase (pRenilla-luc). Luciferase activity was normalized to Renilla luciferase activity, and the firefly luciferase activity in the cell lysate treated with vehicle was set at 1.

Statistical analyses

The data are expressed as the mean ± SEM. Data from the reporter assays and gene expression data were log-transformed to provide an approximation of a normal distribution before analysis. Differences between control cells and cells treated with the

feedstuff extracts were examined using an unpaired *t*-test. Differences of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

To evaluate the transcriptional activity of Pgc-1α, a luciferase-based reporter assay using the Pgc-1α promoter were performed in C2C12 myoblasts; ethanol extracts from 33 feedstuffs were evaluated. The extracts were evaluated at a concentration of 20 μg/mL, except for the red bell pepper extract. Because the red bell pepper extract had a cytotoxic effect in C2C12 cells, the transcriptional activity was examined at a concentration of 2 μg/mL. The reporter assay screen was replicated in at least 3 independent experiments. Representative results are shown in Fig. 1. Luciferase expression was significantly lower in cells treated with oolong tea or roasted green tea extracts of than in control cells. None of the extracts reproducibly increased luciferase expression more than 2-fold (data not shown).

Next, we examined whether the inhibitory effects of tea leaf extract can be detected in the other cells. Pgc-1α transcription is stimulated by forskolin, an activator of adenylate cyclase[17] and by MyoD, a muscle-specific transcription factor.[16] However, Pgc-1α transcription was unaffected by forskolin in COS7 cells, even when MyoD was co-expressed (Fig. 2A). In contrast, HepG2 hepatoma cells were responsive to forskolin irrespective of MyoD expression (Fig. 2A), indicating that Pgc-1α transcription can be evaluated in HepG2 cells. The inhibitory effects of tea leaf extracts on Pgc-1α transcription were weaker in HepG2 cells (Fig. 2B) relative to C2C12 cells (Fig. 1) but were detected in green tea, black tea, roasted green tea and oolong tea extracts. These results suggest that tea leaves contain factor(s) that inhibit Pgc-1α transcription; the activity of the tea leaves is not limited to C2C12 myogenic cells but is also detected in

liver cells. Pgc-1α orchestrates a complex program of metabolic changes that occur during the transition from a fed liver to a fasted liver, including gluconeogenesis; these effects on fasting adaptation are achieved through co-activation of hepatic transcription factors such as hepatic nuclear factor 4α, Pparα, glucocorticoid receptor, Foxo1 and the liver X receptor.[18] Therefore, the tea leaves may also be able to modulate hepatic glucose metabolism.

Effects of tea waste and its silage on Pgc-1α transcription were further evaluated in C2C12 cells (Fig. 3). The tea waste extracts from green tea, black tea and oolong tea did not significantly decrease expression of the Pgc-1α(-2553)-luc construct, but the silage extract effectively repressed Pgc-1α transcription; the effects of oolong tea waste silage were not statistically significant but had a tendency to repress expression (P = 0.08).

Screening feedstuffs using Pgc-1α(-2553)-luc indicates that the extracts from tea leaves and their byproducts cause inhibitory effects on Pgc-1α transcription. We further verified whether these feedstuffs actually down-regulate Pgc-1α expression in myogenic cells; cells were treated during myogenesis with or without the extracts of tea leaves or their byproducts, and Pgc-1α mRNA levels were examined by RT-qPCR. Because C2C12 myoblasts and myotubes did not express Pgc-1α mRNA significantly (data not shown), we used primary myosatellite cells from rat plantaris muscle. Consistent with the reporter assay results (Fig. 1), the oolong tea leaf extract decreased Pgc-1α mRNA expression, whereas cassava starch pulp or dehulled soybean meal extracts did not affect Pgc-1α expression (Fig. 4A). Forskolin treatment increased Pgc-1α expression as expected (Fig. 4A). The extracts from tea waste silage also had the ability to down-regulate Pgc-1α expression (Fig. 4B). Unlike Pgc-1α transcription, Pgc-1α mRNA level was decreased also by the tea waste extract (Fig. 4B); the inconsistent result between the reporter assay and expression at the mRNA level may be

due to 1) transcriptional repression via a region not contained in the Pgc-1α reporter gene or 2) post-transcriptional regulation such as mRNA stability.

The present study identifies tea leaves and their byproducts as negative regulators of Pgc-1α expression. As described above, muscle-fiber type is modulated by the expression level of Pgc-1α; higher expression of Pgc-1α increases the proportion of slow-twitch oxidative muscle-fiber type,[11] whereas the suppression of Pgc-1α expression increases fast-twitch glycolytic muscle-fiber type.[9] Therefore, tea leaves and their byproducts may act as a switch of muscle-fiber types, although future studies should be done to clarify whether they affect muscle fiber types through modulation of Pgc-1α expression in vivo.

Pgc-1α transcription is accelerated through calcium signaling pathway in skeletal muscle; activation of calcineurin stimulates Pgc-1α transcription mediated by activated CREB and MEF2.[18] In addition, Pgc-1α expression is enhanced by glucagon and glucocorticoid signaling in liver.[18] Although mechanisms underlying why tea leaves and their byproducts inhibit Pgc-1α transcription are unclear, the inhibition may be elicited by negative regulation of the transcriptional activation.

Considering that Pgc-1α expression levels in skeletal muscle are positively correlated with the health benefits described above,[8, 9] tea leaves and their byproducts may have a negative effect on skeletal muscle Pgc-1α-mediated improvement of whole-body health in humans. However, the down-regulation of Pgc-1α expression may be beneficial to beef production. Beef color is one important factor in determining the wholesale price of beef in Japan. The dark red color of beef decreases the commodity value of beef in Japan; Japanese consumers prefer lightly colored beef. Muscle-fiber type affects beef color; longissimus muscle and psoas major muscle are a mixture of fast-twitch and

slow-twitch muscle fibers; slow-twitch muscle fibers are reddish, and fast-twitch muscle fiber are whitish. Thus, the tea leaves and their byproducts could potentially enable the production of the preferred color of beef through increased proportion of fast-twitch muscle fiber mediated by the down-regulation of Pgc-1α expression. Furthermore, considering the correlation between slow-twitch muscle fiber content and cooking loss, a beef quality trait,[19] tea leaves and their byproducts may be effective at improving beef quality by decreasing cooking loss. Effects of muscle-fiber type in beef on human nutrition are unknown. Comparison of amino acid composition between fast-twitch myosin and slow-twitch myosin indicates no substantial difference (data not shown).

Canned and bottled tea drinks have become a favorite beverage in Japan. Over 100,000 tons of tea waste per year are emitted from beverage companies; however, much of these waste is burned or composted.[14] Tea waste can be preserved by ensiling due to its high moisture content, thus tea waste silage would be a practical feedstuff to improve beef production.

Down-regulation of Pgc-1α expression by tea leaf byproducts may also lead to an improvement of fattening efficacy, i.e., weight gain per feed intake. Transgenic mice with elevated muscle Pgc-1α expression are resistant to age-related obesity;[8] one of the underlying the events in this process is increased energy expenditure resulting from Irisin production and secretion from the skeletal muscle in response to the upregulation of skeletal muscle Pgc-1α expression.[20] Future studies should identify the specific molecule(s) in the extract of tea leaves and their byproducts that represses Pgc-1α expression, and how these molecule(s) induce transcriptional repression. Considering the abundance of various phenols, polyphenols and tannins in tea leaves,[21, 22] these compounds could be candidates.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by a Grant-in-Aid for Scientific Research (23380158) from The Japan Society for the Promotion of Science and a research project grant awarded by the Azabu University.

REFERENCES

1. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 1998; **92:**829-839.
2. Handschin C, Spiegelman BM. Peroxisome proliferator-activated receptor γ coactivator 1 coactivators, energy homeostasis, and metabolism. *Endocr Rev* 2006; **27:**728-735.
3. Feige JN, Auwerx J. Transcriptional coregulators in the control of energy homeostasis. *Trends Cell Biol* 2007; **17:**292-301.
4. Lin JD. The PGC-1 coactivator networks: chromatin-remodeling and mitochondrial energy metabolism. *Mol Endocrinol* 2009; **23:**2-10.
5. Goto M, Terada S, Kato M, Katoh M, Yokozeki T, Tabata I, Shimokawa T. cDNA Cloning and mRNA analysis of PGC-1 in epitrochlearis muscle in swimming-exercised rats. *Biochem Biophys Res Commun* 2000; **274:**350-354.
6. Baar K, Wende AR, Jones TE, Marison M, Nolte LA, Chen M, Kelly DP, Holloszy JO. Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. *FASEB J* 2002; 16:1879-1886.
7. Norrbom J, Sundberg CJ, Ameln H, Kraus WE, Jansson E, Gustafsson T. PGC-1α mRNA expression is influenced by metabolic perturbation in exercising human skeletal muscle. *J Appl Physiol* 2004; **96:**189-194.
8. Wenz T, Rossi SG, Rotundo RL, Spiegelman BM, Moraes CT. Increased muscle PGC-1α expression protects from sarcopenia and metabolic disease during aging. *Proc Natl Acad Sci U S A* 2009; **106:**20405-20410.
9. Handschin C, Chin S, Li P, Liu F, Maratos-Flier E, Lebrasseur NK, Yan Z, Spiegelman BM. Skeletal muscle fiber-type switching, exercise intolerance, and myopathy in PGC-1α muscle-specific knock-out animals. *J Biol Chem* 2007; **282:**30014-30021.

10. Schiaffino S, Reggiani C. Fiber types in mammalian skeletal muscles. *Physiol Rev* 2011; **91:**1447-1531.
11. Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, Lowell BB, Bassel-Duby R, Spiegelman BM. Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres. *Nature* 2002; **418:**797-801.
12. Czubryt MP, McAnally J, Fishman GI, Olson EN. Regulation of peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) and mitochondrial function by MEF2 and HDAC5. *Proc Natl Acad Sci U S A* 2003; 100:1711-1716.
13. Wu H, Kanatous SB, Thurmond FA, Gallardo T, Isotani E, Bassel-Duby R, Williams RS. Regulation of mitochondrial biogenesis in skeletal muscle by CaMK. *Science* 2002; 296:349-352.
14. Nishino N, Kawai T, Kondo M. Changes during ensilage in fermentation products, tea catechins, antioxidative activity and in vitro gas production of green tea waste stored with or without dried beet pulp. *J Sci Food Agric* 2007; **87:**1639-1644.
15. Asano H, Yamada T, Hashimoto O, Umemoto T, Sato R, Ohwatari S, Kanamori Y, Terachi T, Funaba M, Matsui T. Diet-induced changes in Ucp1 expression in bovine adipose tissues. *Gen Comp Endocrinol* 2013; **184:**87-92.
16. Amat R, Planavila A, Chen SL, Iglesias R, Giralt M, Villarroya F. SIRT1 controls the transcription of the peroxisome proliferator-activated receptor-γ co-activator-1α (PGC-1α) gene in skeletal muscle through the PGC-1α autoregulatory loop and interaction with MyoD. *J Biol Chem* 2009; **284:**21872-21880.
17. Karamitri A, Shore AM, Docherty K, Speakman JR, Lomax MA. Combinatorial transcription factor regulation of the cyclic AMP-response element on the Pgc-1α promoter in white 3T3-L1 and brown HIB-1B preadipocytes. *J Biol Chem* 2009; **284:**20738-20752.

18. Liu C, Lin JD. PGC-1 coactivators in the control of energy metabolism. *Acta Biochim Biophys Sin (Shanghai)* 2011; 43:248-257.
19. Ozawa S, Mitsuhashi T, Mitsumoto M, Matsumoto S, Itoh N, Itagaki K, Kohno Y, Dohgo T. The characteristics of muscle fiber types of longissimus thoracis muscle and their influences on the quantity and quality of meat from Japanese Black steers. *Meat Sci* 2000; 54:65-70.
20. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Boström EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Højlund K, Gygi SP, Spiegelman BM. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012; **481:**463-468.
21. Higdon JV, Frei B. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. *Crit Rev Food Sci Nutr* 2003; **43:**89-143.
22. Clifford MN. Diet-derived phenols in plasma and tissues and their implications for health. *Planta Med* 2004; **70:**1103-1114.

Figure legends

Fig. 1. Inhibition of Pgc-1α transcription by tea leaf extracts in C2C12 cells

C2C12 cells were transiently transfected with Pgc-1α(-2553)-luc and pRenilla-luc, and treated with vehicle or feedstuff extracts for 24 h. The firefly luciferase activity was normalized to the Renilla luciferase activity, and the firefly luciferase activity in cell lysates treated with vehicle was set to 1. Data are expressed as the mean ± S.E. (n = 3). **: $P < 0.01$ *vs.* vehicle. 1: fish meal, 2: glycated rice meal, 3: chicken meal, 4: wine residues, 5: yellow grease, 6: red bell pepper extract, 7: dried distiller's grains with solubles, 8: black soybean branch, 9: cassava starch pulp, 10: skim milk, 11: rapeseed meal, 12: corn gluten meal, 13: black soybean leaf, 14: corn germ meal, 15: wheat flour, 16: shochu distillery by-product, 17: tall fescue, 18: soybean meal, 19: corn gluten feed, 20: yogurt, 21: rice alcohol cake, 22: meat and bone meal, 23: dehulled soybean meal, 24: sake kasu, 25: butter oil, 26: soybean flour, 27: feather meal, 28: wine lees, 29: defatted rice bran, 30: soy protein concentrate, 31: wheat bran, 32: oolong tea, 33: roasted green tea.

Fig. 2. Transcriptional repression of Pgc-1α(-2553)-luc by tea leaf extracts in HepG2 cells

(A) COS7 cells or HepG2 cells were transiently transfected with Pgc-1α(-2553)-luc and pRenilla-luc with or without the MyoD expression plasmid and treated with vehicle or forskolin (10 μM) for 24 h. (B) C2C12 cells were transiently transfected with Pgc-1α(-2553)-luc and pRenilla-luc and treated with vehicle or the indicated extract for 24 h. Firefly luciferase activity was normalized to Renilla luciferase activity, and the firefly luciferase activity in the cell lysates treated with vehicle was set to 1. Data are expressed as the mean ± S.E. (n = 3). **: $P < 0.01$ *vs.* vehicle.

Fig. 3. Transcriptional repression of Pgc-1α(-2553)-luc by extracts of tea waste

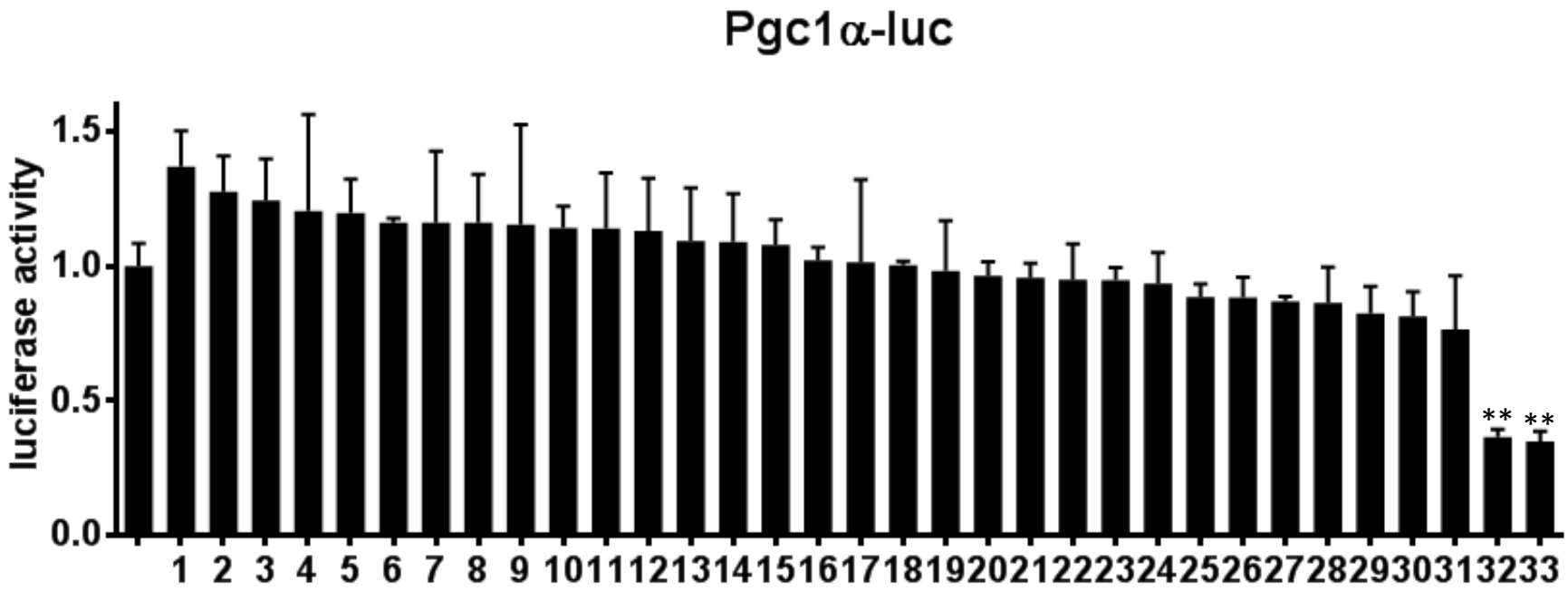
and its silage in C2C12 cells

C2C12 cells were transiently transfected with Pgc-1α(-2553)-luc and pRenilla-luc and were treated with vehicle or the indicated extract for 24 h. Firefly luciferase activity was normalized to Renilla luciferase activity, and the firefly luciferase activity in the cell lysates treated with vehicle was set to 1. Data are expressed as the mean ± S.E. (n = 3). † and **: $P < 0.10$ and $P < 0.01$, respectively, *vs.* vehicle.

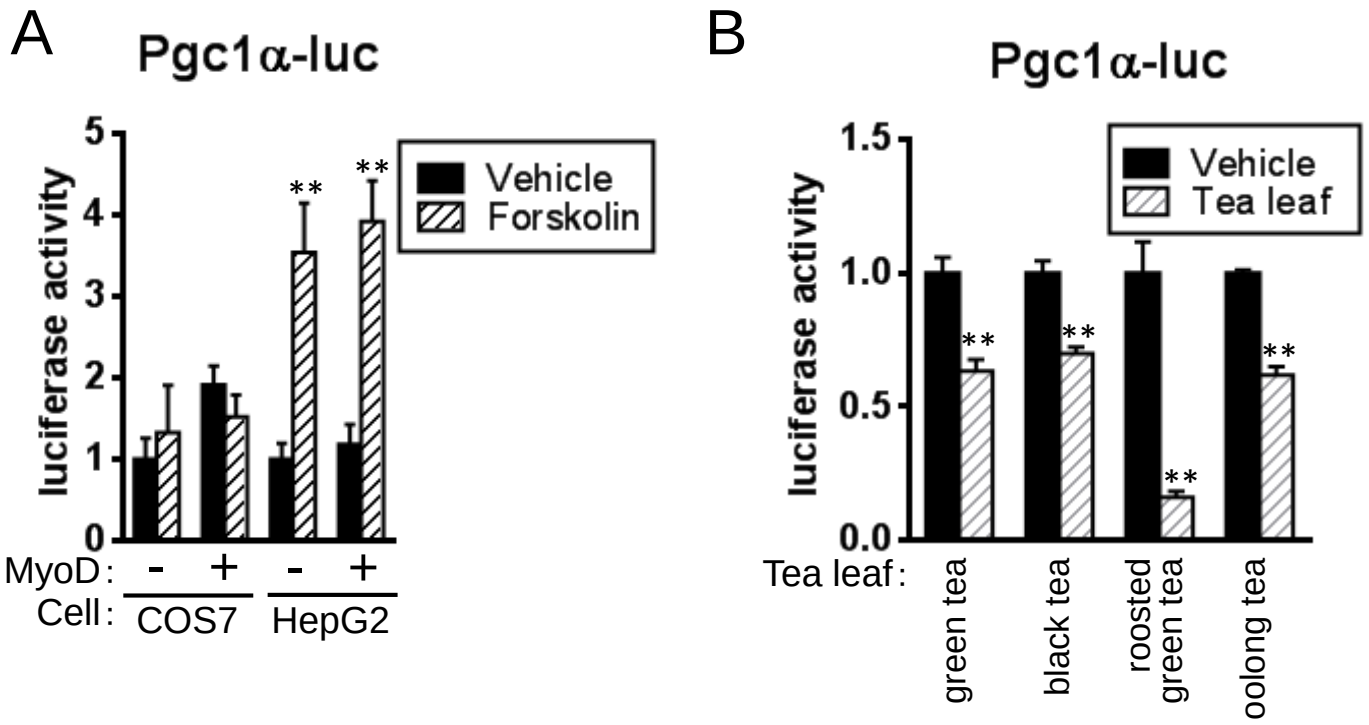
Fig. 4. Down-regulation of Pgc-1α expression by extracts of tea waste and its silage in primary myosatellite cells

Myosatellite cells from rat plantaris muscle were differentiated into myotubes by reducing the serum concentration of the culture medium. After simulating differentiation, the cells were treated with the indicated extract or forskolin 12 days. Pgc-1α mRNA levels were examined by RT-qPCR and normalized to β-actin mRNA levels. The expression level in cells treated with vehicle was set to 1. Data are expressed as the mean ± S.E. (n = 6). **: $P < 0.01$ *vs.* vehicle.

Figure



Figure



Figure

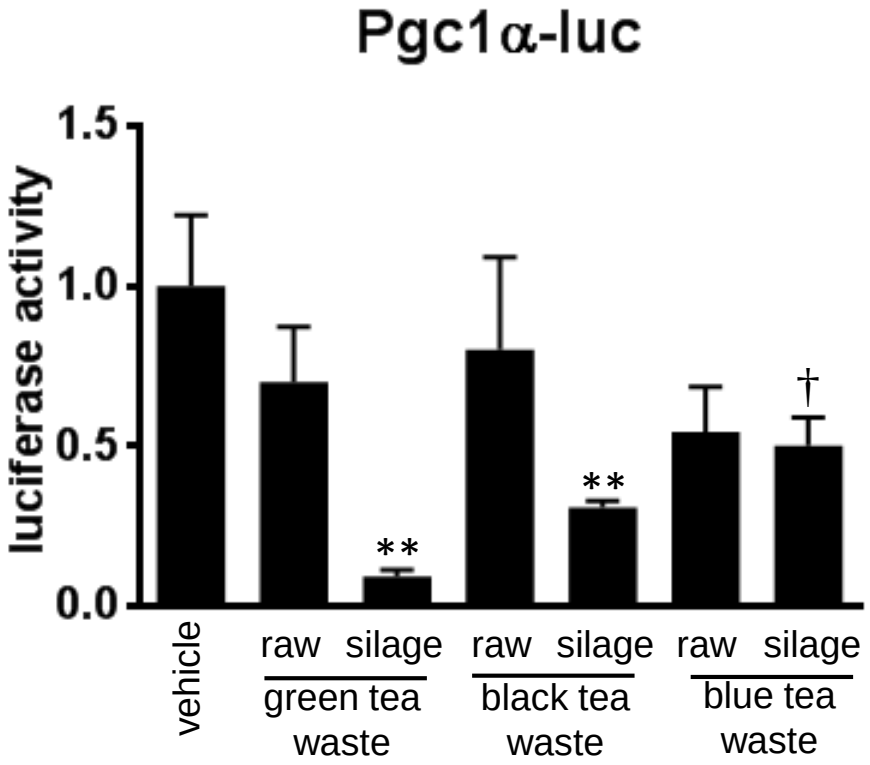


Figure 4